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effects to build upon our understanding of the epidemiology and overall the medical importance of arboviruses.

**Materials and methods:** A multiplex one-step real-time PCR discriminatory for Zika and Chikungunya, with an internal control (IC), was standardized with virus culture. To assess the performance of our assay, it was challenged with clinical samples. From Oct/2015 to Jan/2016, plasma were collected from clinical suspected patients. The RNA was extracted with MDx equipment (QIAGEN), the one-step real-time PCR set up was performed automatically with Janus instrument (PerkinElmer) and the amplification was carried on the sequence detection system QuantStudio (Life Technologies).

**Results:** The assay was adjusted for satisfactory performance, reproducibility, specificity and high sensibility. The IC successfully control all steps and reaction. The threshold line was fixed on 0.3. Eight replicates of ten-fold serial dilution ( $10^{-10}$ ) of Zika and Chikungunya culture virus were analyzed and showed 100% of detection. A total of 43 samples from patients suspected of Zika and 228 of Chikungunya were analyzed. Our assay detected 11 patients positive to Zika and 63 to Chikungunya. Laboratory diagnosis of Zika is challenging, viral RNA can be detected in serum only during the first days of the illness.

**Conclusions:** Molecular techniques offer high sensitivity and specificity, and rapid availability of information. The approach of screening by real-time PCR is well suited for use in a clinical laboratory and can be easily adapted for high throughput screening. The rapid implementation of this Zika/Chikungunya multiplex NAT assay in Brazil will help identify endemic regions and the management of patients.

## P84

### SARS coronavirus has antibody-dependent enhancement (ADE) effect through the autologous antibodies against envelope spikes on Fcγ receptor expressing cells

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**Background:** Severe acute respiratory syndrome coronavirus (SARS-CoV) had an outbreak in 2003. Even though SARS-CoV now remains in the natural bat reservoir, it still has the reemergence threat. Vaccination is a prophylactic strategy for this disease control and prevention. Antibody-dependent enhancement (ADE) is a mechanism by which viruses such as, dengue virus, feline coronavirus, and HIV, as alternative strategies, apply to infect host cells to gain entry into the target cells by taking advantage of anti-viral humoral immune responses. The ADE effect of SARS-CoV infection is controversial.

**Materials:** SARS-CoV TW1 strain (GenBank accession no., AY291451) was obtained from Taiwan CDC and SARS-CoV pseudotyped virus particles harboring the SARS-CoV S protein with HIV core structure virus were constructed for infectivity assay. The anti-SARS CoV infected patient's sera and generated monoclonal and polyclonal antibodies against spike proteins were used for infectivity and neutralization assay. Immunofluorescence staining and transmission electron microscopy observation were performed to assay the viral susceptibility and ADE on HL-CZ cells with SARS-CoV infection in the presence of the different dilutions of anti-sera against SARS-CoV.

**Results:** We found that SARS-CoV uses ADE to enhance its infectivity towards a human promonocyte cell line-HL-CZ. Quantitative-PCR and immunofluorescent staining indicated that SARS-CoV can replicate in HL-CZ and display virus-induced cytopathic effect as well as increased TNF- $\alpha$ , IL-4 and IL-6 two days postinfection. Results from flow-cytometry indicate HL-CZ cells express angiotensin converting enzyme 2 (ACE2), a SARS-CoV receptors and higher level of FcRRII receptors.

Our data demonstrated that higher diluted sera from SARS-CoV infection patients promote SARS-CoV infection and induced higher level of apoptosis. Infectivity assay demonstrated that ADE of SARS-CoV is majorly mediated by diluted antibodies against envelope spikes rather than nucleocapsid proteins. We further generated monoclonal antibodies against spike proteins of SARS-CoV and found that most monoclonal antibodies promote SARS-CoV infection.

**Conclusions:** We suggested that antibodies against spike proteins of SARS-CoV may cause ADE effect. This data raises reasonable concern regarding the use of SARS-CoV vaccine and shed light on some roles in SARS pathogenesis.

## P85

### Severe dengue type one outbreak in Taiwan in 2014

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**Introduction:** Dengue epidemic has been circulating in southern Taiwan, especially in Kaohsiung city. A severe dengue fever (DF) outbreak reported in Taiwan in 2014. Notably, an accident underground pipeline explosion was occurred in the midnight of 1st August and subsequently accompanied with continuous rain. We hypothesized that underground explosion is an important factor to trigger this outbreak.

**Materials and methods:** We collaborated with Kaohsiung Medical University Hospital system to collect 4,400 serum samples from DF patients. The virus isolation was conducted using C6/36 insect cells. RT-PCR and phylogenetic analyses using NS1 and Env gene for serotyping and genotyping respectively. The information of the DF and dengue hemorrhagic fever (DHF) was collected from Taiwan CDC. The data of precipitation, temperature changes and consecutive raining days in Kaohsiung were offered by Taiwan Central Weather Bureau.

**Results:** The data indicate that there were 15,732 reported cases of DF in 2014, including 15,492 indigenous cases and 240 imported cases. The mass of DF cases were reported in Kaohsiung City (15,043 cases; 95.6%). Results showed that after gas explosion, the percentage of Breatou index (BI) to be above 5–9% (level II) for the measure of mosquito density increasing from 36% to 48% and the DF cases also increased dramatically in 1–2 months after the gas explosion. Phylogenetic tree analysis showed that all the outbreak-associated dengue viruses were belonged to serotype 1 and subgenotype E4. Climate changes have a significant impact on the transmission and incidence of DF. We found rainfall and higher temperature significantly correlate with 2014 dengue outbreak (Spearman correlation analysis;  $P < 0.0001$ ;  $r = 0.87$  and  $0.78$ , respectively). Results also indicate that earlier trends of imported DF occurrences significantly caused the later indigenous epidemics or outbreaks during 2005–2014 (Spearman correlation analysis;  $P < 0.0001$ ). We found that the most dengue epidemics in Taiwan sourced from our neighboring countries.

**Conclusions:** We conclude that three main factors that may have been associated with this DF outbreak in Kaohsiung: an underground pipeline explosion combined with subsequent rainfall and higher temperature. These factors may have enhanced mosquito breeding activity, facilitating DENV transmission.

## P86

### Identification of Dengue and Chikungunya viruses among suspected cases of Yellow fever in the Democratic Republic of Congo

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**Introduction:** No etiological exploration is ever done for more than 99% of acute febrile jaundice identified through the surveillance of yellow fever. Our goal was to investigate other arthropod-borne viruses